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Assessment of the risk of botulism from chilled, vacuum/modified atmosphere packed fresh beef, lamb and pork held at 3 °C–8 °C

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ABSTRACT

The safety of current UK industry practice (including shelf-life) for chilled, vacuum/modified atmosphere-packed fresh red meat (beef, lamb and pork) held at 3°C–8°C has been evaluated with respect to non-proteolytic *Clostridium botulinum*. UK industry typically applies a retail pack shelf-life at 3°C–8°C to 13 days for fresh red meat, with a maximum of 23 days for beef, 27 days for lamb, and 18 days for pork. An exposure assessment established that current commercial practice for fresh red meat provided strong protection with more than 10^{10} person servings marketed in the UK without association with foodborne botulism. A challenge test demonstrated that spores of non-proteolytic *C. botulinum* inoculated on chilled vacuum-packed fresh red meat did not lead to detectable neurotoxin at day 50 for beef, day 35 for lamb, or day 25 for pork (i.e. < 40 pg type B toxin and type E toxin g^{-1} of meat). The products were visually spoiled many days before these end points. The exposure assessment and challenge test demonstrated the safety of current UK industry practices for the shelf-life of fresh, vacuum-packed beef, lamb and pork held at 3°C–8°C with respect to *C. botulinum*, and that botulinum neurotoxin was not detected within their organoleptic shelf-life.

1. Introduction

The botulinum neurotoxin is the most potent toxin known, with a human lethal dose potentially as low as 30 ng (Peck, 2006, 2009; Lindström et al., 2009). The neurotoxin is formed by several clostridia (notably *Clostridium botulinum*) and possibly also by a strain of *Enterococcus* (Peck et al., 2017; Brunt et al., 2018; Smith et al., 2018; Zhang et al., 2018). *C. botulinum* is a heterogeneous grouping of four discrete anaerobic spore-forming bacteria (*C. botulinum* Groups I to IV), that are sufficiently distinct as to be considered separate species (Peck, 2006, 2009; Lindström et al., 2009). Foodborne botulism is a severe intoxication caused by the consumption of food containing pre-formed botulinum neurotoxin, most frequently formed by a strain of *C. botulinum* Group I or II. *C. botulinum* Group I (proteolytic *C. botulinum*) is a mesophilic bacterium with a minimum growth temperature of 10°C–12 °C, while *C. botulinum* Group II (non-proteolytic *C. botulinum*) is a psychrotrophic bacterium with a minimum growth temperature of 3 °C. The ability to form neurotoxin at chilled temperatures makes *C. botulinum* Group II (non-proteolytic *C. botulinum*) a concern for the safe production of chilled foods (Lindström et al. 2006a; Peck, 2006, 2009; FSA, 2017). Spores formed by non-proteolytic *C. botulinum* are present in the environment and may contaminate chilled fresh meat. In the absence of controlling factors (e.g. competition from other micro-organisms), spores of non-proteolytic *C. botulinum* may germinate, leading to cell multiplication and neurotoxin formation during the storage of chilled fresh meat to a level that is detrimental to human health.

Vacuum packaging (VP) and low-oxygen modified atmosphere packaging (MAP) are widely used by the food industry in the production of chilled foods (including fresh meat) and may contribute to extend shelf-life. The presence of no/little oxygen may be particularly conducive to growth and formation of botulinum neurotoxin by the anaerobic bacterium non-proteolytic *C. botulinum*. Interestingly, in some circumstances, toxin formation by non-proteolytic *C. botulinum* can be as rapid in foods packed in air as under VP or low-oxygen MAP, presumably because the food can be itself reduced (Peck et al., 2008). The mean redox potential within beef and pork loins stored chilled for 0 and 7 days was *ca.* −140 mV when vacuum packed and −70mV when aerobically packed (Kim et al., 2002); not sufficient alone to restrict growth from spores of non-proteolytic *C. botulinum* (Lund and Wyatt, 1984; Lund et al. 1984).

A limited number of risk assessments have been carried out for *C. botulinum* (e.g. Barker et al., 2002, 2005; Malakar et al., 2011; Membre et al., 2015). However, exposure assessments have been used to

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estimate the level of protection provided by current practice for specific foods with respect to C. *botulinum* (Hauschild and Simonsen, 1985, 1986; Peck et al. 2006). Hauschild and Simonsen (1985, 1986) considered proteolytic *C. botulinum* and shelf stable meats and noted that although there were few published datasets (e.g. challenge test data), a safety assessment could be made using industry data on the numbers of individual packs of product produced over many years, together with recorded cases of botulism attributed to those products. This provided a meaningful assessment of actual risks from those products in the past, and if existing commercial practices were described and have not changed, then this could show that the controls used were valid and lead to a safe product. For various defined luncheon meats, canned cured ham and sausages, it was estimated that $> 10^7$ to $> 10^9$ products had been sold without reported incidence of botulism (Hauschild and Simonsen, 1985, 1986). Peck et al. (2006) used the same approach for non-proteolytic *C. botulinum* in chilled foods, including MAP and VP fresh meat, with a maximum shelf-life of 10 days at ≤8 °C and no specific single controls (pH, water activity, salt etc.), other than storage temperature and shelf life. It was established that 8.3×10^9 ($10^{9.8}$) packs of chilled food had been sold with a maximum shelf-life of 10 days at ≤8 °C between 1986 and 2005, with no associated cases of foodborne botulism (Peck et al., 2006). These findings led to the adoption of the 10-day rule for chilled foods in the UK (i.e. maximum 10-day shelf life at ≤8 °C) (ACMSF, 2006). Important qualifications to this approach include the assumption that the existing commercial and consumer practice remains unchanged, and that although the probability of botulism incidents remaining undetected is low, reporting of the illness may not be complete.

The aim of this study has been to evaluate the safety of current UK industry practice (including shelf-life) for chilled, VP/MAP fresh red meat (beef, lamb and pork) held at 3 °C–8 °C, with respect to non-proteolytic *C. botulinum*. The first objective has been to carry out an exposure assessment to determine the level of protection when employing current commercial practice regarding VP/MAP fresh red meat that does not contain known controlling factor(s). The second objective has been to conduct a challenge test experiment with non-proteolytic *C. botulinum* and fresh chilled red meat representative of that sold in the UK. For the purpose of this study, fresh red meat is taken to mean meat that has not undergone any preserving or other process except chilling, freezing or quick-freezing, including meat that is VP or MAP wrapped (EC, 2004). The study focussed on beef, lamb, and pork, as these are the three species of fresh meat for which a significant number of portions have been consumed in the UK over the last decade or more.

2. Materials and methods

2.1. Data collection from literature and other sources

Literature searches were carried out of; (i) prevalence of spores of non-proteolytic *C. botulinum* types B, E or F in meat, (ii) outbreaks of foodborne botulism involving chilled foods sold commercially, and (iii) data on neurotoxin formation and growth of non-proteolytic *C. botulinum* on fresh red meat. Records were retrieved from online databases (Web of Science, PubMed, Google Scholar), then combined with articles held in personal literature collections and references cited in/or citing eligible articles. Searches were not restricted by country or language. References were checked for suitability based on a review of titles and abstracts, which identified a sub-set for which the entire article was then individually assessed for relevance, and appropriate information extracted. Additional information on foodborne botulism outbreaks in France was provided by colleagues at Institut Pasteur (Paris).

Details of current industry practice were established through consideration of legislation and specifications, and data provided by industry members of the project consortium. Market sales data were derived through analysis of data from internationally recognised sources.

2.2. Protocol for challenge test experiment

The challenge test was carried out in accordance with a practice developed in conjunction with industry (Anon, 2018) that reflected peer reviewed methods used previously and was consistent with the approach recommended and used by others internationally (Doyle, 1991; NACMCF, 1992, Health Canada Food Directorate, 2010; NACMCF, 2010). Challenge test guidance documents produced in the USA/Canada emphasise the importance of verifying that neurotoxin formation can be prevented (Doyle, 1991; NACMCF, 1992, Health Canada Food Directorate, 2010; NACMCF, 2010). The experimental protocol was finalised through discussions with industry members of the project consortium.

Spores were produced of twelve strains of non-proteolytic *C. botulinum* that formed a good quantity of botulinum neurotoxin. There were seven type B strains (all sub-type B4; Eklund 2 B, Eklund 17 B, CDC 3875, Prevot 59, Kapchunka B2, IFR 05/20, IFR 05/25) and five type E strains (sub-types E1, E2, E3; Beluga, Hazen 36208, Prevot P34, Dolman VH, CDC 8073). These strains were selected to reflect the genetic/physiological diversity of non-proteolytic *C. botulinum* and are commonly used in challenge tests (Stringer et al., 2013; Peck and van Vliet, 2016). The spores were produced and washed free of toxin using standard methods, and enumerated by viable counts (Peck et al., 2010). The spore cocktail was prepared in 0.85% saline and contained an equal number of spores of each strain, and the final concentration of spores added to the meat was confirmed by viable count.

Six examples of fresh meat (two each of beef, lamb and pork) were tested (Table 1), each from a different supplier. For each meat species, meat from a short maturation time (SM) and a long maturation time (LM) were tested. These were selected by the British Meat Processors Association (BMPA) to reflect the UK market. Following slaughter, the meat was held at < 3 °C prior to shipping, during shipping, and prior to inoculation. The meat samples tested were approximately 125 g (Table 1); with this mass selected to reflect the lowest mass sold commercially. The initial pH of each meat was measured after the meat sample had been homogenised with an equal mass of distilled water (Table 1).

For inoculation, each bag of meat was opened, and 2×100 µl of the heat activated (70 °C/10 min) spore cocktail was surface inoculated onto the meat to a final concentration of 500 spores per bag (4 spores/g meat). The spore inoculum was plated to confirm that the correct inoculum had been added. The spore inoculum concentration was selected to be both conservative and more representative of natural contamination than that used in some previous challenge tests with fresh meat. Barker et al. (2016) provided a quantification of non-proteolytic *C. botulinum* spore loads in various food materials, including meat. For most food materials, the spore loads were expected to centre on a concentration range of 1–10 spores kg^{-1}, with a lower contamination of meat. In meat, the probability of the spore loading exceeding 10 spores kg^{-1} was estimated as 2×10^{-7} (Barker et al.,

Table 1
Summary of fresh chilled meat samples used in the challenge test experiment.

Sample code	Maturation time[a]	Mass (g) of meat per pack [mean ± Std Dev]	Mean initial pH of meat
Beef SM	7 days	118 ± 6	5.53
Beef LM	54 days	127 ± 3	5.57
Lamb SM	8 days	131 ± 5	5.71
Lamb LM	64 days	123 ± 3	5.76
Pork SM	5 days	117 ± 5	5.68
Pork LM	23 days	121 ± 4	5.46

[a] Maturation time = number of days between animal slaughter and challenge test inoculation. During this time the meat was held at < 3 °C in chilled primal and/or retail packs. SM = short maturation time, LM = long maturation time.

2016). A literature search identified reports of additional tests to those described in Barker et al. (2016) for spores of non-proteolytic *C. botulinum* in meat (Sathish and Swaminathan, 2009; Chukwu et al., 2016; Grenda et al., 2017), all of which were negative. Therefore, this increases the belief that small spore loads are to be expected in fresh meat at the expense of beliefs about large loads.

The inoculated bags were then vacuum packed, and gently massaged to distribute the spores. Uninoculated packs of each meat served as the negative control. Anaerobic microbiological broth (Robertson's cooked meat medium) inoculated with the spore cocktail served as the positive control. A conservative approach for temperature storage was used. All inoculated/uninoculated meat samples and microbiological broth were incubated at < 3 °C for 1 day (measured temperature (mean ± standard deviation) = 1.7 °C ± 0.1 °C), then at 5 °C for 1 day (measured temperature = 4.9 °C ± 0.1 °C) to reflect commercial distribution practice, then at 22 °C for 2 h to simulate potential abuse during consumer purchase and transportation, and finally at 8 °C (measured temperature = 8.0 °C ± 0.1 °C) for the remaining incubation period to reflect domestic storage. In the reported results, day 0 is the last day at < 3 °C, day 1 is after 24 h at 5 °C, day 2 is after 24 h at 8 °C etc.

Triplicate packs of inoculated and uninoculated meat (and duplicate bottles of positive control microbiological broths) were removed at each of eight sampling times (nine for beef) and observed for signs of visual spoilage. Samples were frozen on the day of sampling, thawed later, and tested for the presence of botulinum neurotoxin type B and type E. Botulinum toxin was extracted from the entire sample using gelatine phosphate buffer (total dilution 1:4). Tests for botulinum toxin were carried out in a type B toxin ELISA and type E toxin ELISA similar to those described previously (Ferreira, 2001; FDA (Food and Drug Administration), 2001; Peck et al., 2010). Sample extracts were tested in duplicate wells in the type B ELISA and type E ELISA, and the mean absorbance of each test sample compared to a standard curve constructed using a neurotoxin standard in the same meat extract on the same ELISA plate. Test samples were considered positive for botulinum neurotoxin if the mean absorbance was greater than the absorbance given by 40 pg botulinum neurotoxin g^{-1} meat in the type B ELISA or type E ELISA. This detection limit is comparable to that achieved using the mouse bioassay (Anon, 2018). The detection limit is several orders of magnitude lower than the human lethal oral dose of botulinum toxin (approximately 30 ng).

3. Results

3.1. Current UK industry practice

Current UK industry practice established for beef, lamb and pork from carcass to end of fresh meat shelf life, includes statutory (EC, 2004) and commercially-required controls to minimise potential for contamination from slaughter, and *C. botulinum* toxin formation at various points in the supply chain. Fresh meat is usually sold either as full carcasses, animal sections (primals) or smaller cuts such as steaks and chops. Fresh meat is also purchased from abattoirs and processors by both wholesalers and large foodservice businesses. Foodservice is an often overlooked but still vital part of the meat industry.

In the UK market, most sales of fresh beef, lamb, and pork are through retailers. As a condition of trading, UK retailers and their suppliers require compliance with relevant legislation and complementary industry technical standards. These include UK Fresh Meat Hygiene Regulations (FMHR, 1995), EU Regulation 853/2004 (EC, 2004), UK FSA Meat Industry Guide (FSA, 2018), BMPA codes, and assurance schemes recognised by BMPA and major UK retailers (such as: BRC Global Standard (Food Safety, Storage and Distribution), Red Tractor (beef and lamb) and Quality Meat Scotland). Each follows EU legislated requirements, and add features in relation to quality, welfare and/or provenance, and is audited against. While each scheme differs, they are not in conflict with legislation. In recent years, there have been developments in the ability to produce a vacuum and to precisely modify the gas composition in a sealed packet.

Once the meat is cut, and where appropriate packaged, the meat must be chilled to no more than 3 °C (offal) and 7 °C (other meat). Specifications for the temperature of fresh meat on receipt for further processing was confirmed by industry to range from 3 to 5 °C. Maturation of primals prior to retail packing is most frequently wet maturation where the meat is not directly exposed to air, but is in a sealed no/low oxygen pack and normally VP. Dry maturation, where meat is directly exposed to air, also known as dry-ageing, is becoming more widespread in the UK retail fresh beef market. Maturation times typically range from 0 to 42 days for beef, 0–77 days for lamb, and 0–21 days for pork, with longer times possible for beef and lamb from Australia/New Zealand. Maturation temperature profiles provided by project consortium members indicated a general adherence to storage core temperatures of 0–3 °C, although storage at > 3 °C may occur for very limited periods. Storage at < 3 °C does not have a temporal limit with respect to non-proteolytic *C. botulinum* (as this temperature suppresses toxin formation), but other factors such as organoleptic quality of the meat may need to be considered. VP/MAP primals are distributed widely, including to manufacturers of further processed products where specifications require receipt at no more than 3–5 °C, to foodservice operations, to independent butchers, and to some retailers using vertically integrated supply chains and carrying out cutting operations in-store. In the UK the commercial storage of chilled foods must comply with legislation by country (FSA, 2016). In England, Wales and Northern Ireland chilled food must be kept at a temperature of 8 °C or below (FSA, 2016). In Scotland, there is not a specific chilled storage temperature, but official guidance (FSA, 2016) recommends that if the food storage place chosen exceeds 8 °C then the shelf life of the foodstuff may need to be reduced.

Overall, data on temperatures, holding times and shelf lives comprising industry practice in relation to UK retail were relatively consistent. The only divergences appear to be that individual companies might have a different retail shelf life for different cuts of one type of meat, and that different companies might have a different retail shelf life for the same cut of one type of meat. Currently, UK industry as represented by the project consortium typically applies a chilled retail shelf life of up to 11–13 days to packs of fresh beef, lamb, and pork, with a maximum of 18–27 days (Table 2).

Although consumer packs of fresh meats are kept chilled in the commercial distribution chain, their useful shelf life is relatively short. This is due to the lack of deep chill during storage by consumers. Without deep chill there is little protection against microorganisms that cause meat spoilage. Temperatures of domestic refrigerators can be influenced by appliance type and vary from front to back and top to bottom. In 2008, it was reported that the overall mean temperature was < 5 °C for 28% of UK domestic fridges, with a mean temperature of 6.6 °C (Peck et al., 2008). A recent survey of 671 domestic refrigerators in England found the overall mean temperature to be 5.3 °C, with a considerable proportion exceeding 5 °C throughout the study period (Biglia et al., 2018).

Shelf life is assured in practice by applying high hygiene standards during production, including sourcing of the animals to result in low spore loadings (Barker et al., 2016), of low distribution and storage

Table 2
Minimum, typical and maximum UK retail shelf lives for fresh beef, lamb, and pork.

	Beef	Lamb	Pork
Minimum	7 days	7 days	7 days
Typical	8–13 days	8–11 days	8–11 days
Maximum	23 days	27 days	18 days

temperatures to the point of purchase by the consumer, and limitation of shelf life. The water activity and pH of fresh meat are as a rule non-controlling in relation to non-proteolytic *C. botulinum*. For established products, shelf life is based on the history of the product and its composition. The pattern of VP widespread usage for primals and joints worldwide is largely similar to the UK, with a particular emphasis on small producers for VP end products. Shelf lives outside the UK are reported to be similar to those in the UK.

3.2. Market sales data

The number of portions of fresh red meat (beef, lamb and pork) was based on each person serving being a portion of 250 g. The derivation of this mass was conservative and reflected evidence. Meat & Livestock Australia (MLA) reported that 163 g raw meat was the mean mass consumed in Australia (MLA, 2012). UK National Diet and Nutrition Survey consumption data (NDNS, 2018) referred to 'Red and processed meat' which included ham, bacon and other meat-based items. For UK data going back to the 2008-9 NDNS survey, the 97.5 percentile highest daily consumption of red and processed meat was 236 g, which was by men 19–64 years old in 2008–9.

The pre-packed chilled fresh meat sector has existed for some 50 years in industrialised countries. A majority of chilled pre-packed fresh meat products are MAP or VP. Fresh meat primals tend to be VP throughout the world. VP coupled with deep chill, is the basis of the meat export industry. Two datasets have been consolidated to establish the UK consumption of fresh beef, lamb and pork between 1999 and 2017 (except for 2006). Market data for 1999–2005 were summarised in 2006 (Peck et al., 2006). However, the number of portions were not specified, and these have been calculated based on the mass of meat sold. It is estimated that 7.8×10^9 250 g portions of fresh beef, lamb, and pork were sold in the UK between 1999 and 2005 (Table 3). BMPA data (BMPA, 2018) were used to establish that UK sales of beef, lamb and pork through major supermarket chains for 2007–2017. For these three species, the total mass consumed in the UK sold through the major multiples was 1.0×10^7 tonnes, and the number of portions consumed in the UK sold through the major multiples was 4.1×10^{10} 250 g portions. Correcting for retailers' 76% market share (Peck et al., 2019), then from 2007 to 2017 the total mass consumed in the UK was 1.3×10^7 tonnes, and the number of portions consumed in the UK was 5.4×10^{10} 250 g portions (Table 3). Consolidating the datasets from 1999 to 2005 and 2007–2017 gives combined total UK sales figures (of 250 g portions) of beef, lamb and pork of 3.1×10^{10}, 8.6×10^9 and 2.2×10^{10}, respectively. This is a total of 6.2×10^{10} 250 g portions (Table 3).

FAO (FAO, 2018) reported on consumption of beef, lamb and pork from 1964 to 2015. Assuming a global population of 7.52×10^9 (Population pyramid, 2018), it can be calculated that 8.3×10^{11} 250 g portions of meat were consumed globally in 2015 (Table 4). Note that caprine meat has been included as lamb. Combining Organisation for Economic Co-operation and Development (OECD) data for global consumption per capita of beef, lamb and pork in 2017 (OECD, 2018) with the above estimate of the global population, then the global consumption of beef, lamb and pork in 2017 totalled 6.2×10^{11} 250 g portions (Table 4). This includes 9.3×10^{10} 250 g portions in EU28. However, it must be noted that not all the presentations will have been as fresh chilled meat and/or VP/MAP, although where fresh chilled meat is handled, the established technology for managing it prior to further handing is VP, particularly in well-developed countries. These two estimates indicate significant annual global consumption of fresh beef, lamb, and pork.

Table 4
Global number of 250 g portions of Fresh Beef, Lamb, and Pork Consumed in 2015 and 2017.

Meat	Total number of portions consumed	
	2015 (FAO data)	2017 (OECD data)
Beef	3.0×10^{11}	2.0×10^{11}
Lamb	6.3×10^{10}	5.2×10^{10}
Pork	4.6×10^{11}	3.7×10^{11}
Total	8.3×10^{11}	6.2×10^{11}

3.3. Incidents of foodborne botulism involving commercial foods intended to be stored chilled

A literature review identified 26 botulism outbreaks associated with commercial foods intended to be stored chilled; taken as a worse-case scenario for fresh chilled meat (Table 5). Sixteen outbreaks were associated with proteolytic *C. botulinum*, one with *C. baratii*, and four with non-proteolytic *C. botulinum* (all type E toxin in vacuum-packed fish). In a further five outbreaks, the organism responsible for causing botulism was not reported, including three outbreaks caused by type B toxin (Table 5), and it is not clear whether proteolytic *C. botulinum* or non-proteolytic *C. botulinum* strains were involved. The dominance of proteolytic *C. botulinum* highlights the significance of temperature abuse. Importantly, however, none of the 26 botulism outbreaks implicated correctly stored commercially-prepared chilled foods; illness occurred when foods were time and/or temperature abused or when pre-formed botulinum toxin was inadvertently added, via another food component, to a correctly chilled product (Table 5). Thus, commercially produced foods intended to be stored chilled do not appear to have been implicated in foodborne botulism when the shelf-life and storage temperature have been maintained as specified by the manufacturer. The lack of reported associated outbreaks suggests that current practice leaves a good safety margin. However, while outbreaks of foodborne botulism are frequently severe and deadly, mild cases of botulism are also described (Rao et al., 2018), and it is possible that under-reporting may lead to some outbreaks of foodborne botulism being unrecognised.

Meat products (family or artisan prepared) are associated with foodborne botulism in France, with commercial meat products also implicated in several outbreaks between 1998 and 2016 (Carlier et al. 2001, 2007; Haeghebaert et al. 2003; Mazuet et al. 2011, 2014, 2018). In outbreaks involving commercial sausages in 2003 (saucisson) and 2013 (chorizo), the sausages were intended to be stored at ambient

Table 3
UK sales of fresh beef, lamb, and pork (1999–2005 and 2007–2017).

Meat	UK sales from 1999 to 2005[a]		UK sales from 2007 to 2017[b]		Total UK sales 1999–2005 and 2007–2017	
	Total tonnes	Total no. portions	Total tonnes	Total no. portions	Total tonnes	Total no. portions
Beef	5.6×10^5	2.2×10^9	7.2×10^6	2.9×10^{10}	7.8×10^6	3.1×10^{10}
Lamb	2.1×10^5	8.4×10^8	2.0×10^6	7.8×10^9	2.2×10^6	8.6×10^9
Pork	1.2×10^6	4.8×10^9	4.3×10^6	1.7×10^{10}	5.5×10^6	2.2×10^{10}
TOTAL	2.0×10^6	7.8×10^9	1.3×10^7	5.4×10^{10}	1.6×10^7	6.2×10^{10}

[a] Peck et al. (2006).
[b] BMPA data (BMPA, 2018) corrected for retailers' 76% market share (Peck et al., 2019).

Table 5
Examples of foodborne botulism outbreaks involving commercial foods intended to be stored chilled.

Country (year)	Product	Organism (toxin type)	Cases (deaths)	Factors contributing to outbreak	References
Canada (1985)	Garlic-in-oil	Prot (B)	36	No preservatives; temperature abuse	St. Louis et al. (1988)
UK (1989)	Hazelnut yoghurt	Prot (B)	27(1)	Toxin added with canned hazelnut conserve to correctly chilled yoghurt	O'Mahony et al. (1990)
USA (1989)	Chopped garlic-in-oil	Prot (A)	3	Temperature abuse (product not refrigerated)	Morse et al. (1990)
USA (1990)	Grilled fresh Palani (surgeon fish)	NR (B)	3	Temperature abuse	CDC (1991)
USA (1993)	Canned cheese sauce (restaurant)	Prot (A)	8(1)	Contamination of canned cheese sauce after opening, then temperature abuse (opened tin not refrigerated)	Townes et al. (1996)
USA (1994)	Potato dip (“skordalia”) and aubergine dip (“meligianoslata”) (restaurant)	Prot (A)	30	Toxin added with temperature-abused baked potatoes to correctly chilled yoghurt dishes	Angulo et al. (1998)
USA (1994)	Clam chowder	Prot (A)	2	Temperature abuse (product not refrigerated)	Sobel et al. (2004)
USA (1994)	Black bean dip	Prot (A)	1	Temperature abuse (product not refrigerated)	Sobel et al. (2004)
Canada (1995)	Country-style pâté	NR (B)	2	Temperature abuse (product not refrigerated)	Leclair et al. (2013)
Italy (1996)	Mascarpone cheese	Prot (A)	8(1)	Temperature abuse; pH > 6	Aureli et al. (2000)
Germany (1997)	Hot-smoked, vacuum-packed fish	NP (E)	2	Suspected temperature abuse	Korkeala et al. (1998)
Argentina (1998)	Meat roll (“matambre”)	Prot (A)	9	Insufficient cooking, lack of preservatives, vacuum-packed in heat-shrunk plastic, & inadequate refrigeration	Villar et al. (1999)
France (1999)	Fish soup	Prot (A)	1	Temperature abuse at home	Carlier et al. (2001)
Canada (2001)	Cooked boneless pork product	Prot (A)	1	Temperature abuse (product not refrigerated)	Leclair et al. (2013)
Germany (2004)	Vacuum-packed smoked salmon	NP (E)	1	Consumed 3 days after “use by date”	Dressler (2005)
UK (2004)	Organic hummus	NR	1	Time/temperature abuse	McLauchlin et al. (2006)
Canada/USA (2006)	Refrigerated carrot juice	Prot (A)	6	Temperature abuse; product pH between 6 and 7	Sheth et al. (2008)
Finland (2006)	Vacuum-packed smoked whitefish	NP (E)	1	Suspected temperature abuse	Lindström et al. (2006b)
China (2007)	Sausages	Prot (A)	66	Temperature abuse (product not refrigerated)	Zhang et al. (2010)
France (2008)	Chicken enchiladas	Prot (A)	2	Time/temperature abuse (product not refrigerated); consumed 1 day after “use by date”	King & the French Multidisciplinary Outbreak Investigation Team (2008)
France (2009)	Vacuum packed hot-smoked whitefish	NP (E)	3	Suspected temperature abuse during travel and home storage	King et al. (2009)
Italy (2010)	Cream of vegetable soup	NR (B)	1	Temperature abuse (product not refrigerated); long shelf-life	Daminelli et al. (2011)
USA (2011)	Potato soup	Prot (A)	2	Temperature abuse (product not refrigerated)	CDC (2011)
New Zealand (2015)	Chilled, ready-to-eat risotto	NR	1	Time/temperature abuse (product not refrigerated; consumed several months past best-before date)	Smyth et al. (2015)
Slovakia (2015)	Hummus spread	Prot (A)	1	Suspected temperature abuse	Mad'arova et al. (2017)
France (2015)	Frozen minced beef used in restaurant Bolognese sauce	*C. baratii* (F)	3	Time/temperature abuse (sauce prepared ≥24 h in advance, left at room temperature for several hours)	Mazuet et al. (2017)

Prot: proteolytic *C. botulinum*, NP: non-proteolytic *C. botulinum*, NR: not reported.

temperature. A single case in 2012 was suspected to involve commercial pâté from Bulgaria, but product details are not available. A single botulism case in 2015 may have involved commercial jambon blanc intended to be stored chilled, although the origin of this botulism case is not clear, as a strain of non-proteolytic *C. botulinum* type B was found in a sample of jambon blanc and the patient stool, but botulinum neurotoxin was not detected in the product. In summary, no outbreaks of botulism have been confirmed in France with commercial industrial chilled products when the shelf-life and storage temperature have been respected.

3.4. Exposure assessment

Previous exposure assessments have used data on the number of individual packs of product produced over many years, together with recorded cases of botulism attributed to those products, to calculate the level of protection. This has provided a meaningful assessment of actual risks from those products in the past, and as long as existing commercial and consumer practices are described and remain unchanged, then this information can be used to show that the controls in place are valid and have led to a safe product.

Existing commercial practice in the UK for chilled, VP/MAP fresh meat (beef, lamb, and pork) held at 3 °C–8 °C provides a high level of protection with respect to non-proteolytic *C. botulinum*. Total UK sales of fresh chilled beef, lamb, and pork between 1999-2005 and 2007–2017 amounted to 6.2×10^{10} ($10^{10.8}$) portions each of 250 g, with no associated cases of foodborne botulism. Existing commercial and consumer practices have been described and are reported to be unchanged during this period. It is important to note some of the underlying uncertainties and assumptions, for example: (i) foodborne botulism associated with commercial chilled foods is not under-reported; (ii) described existing commercial practices remain unchanged, e.g. fraction sold with typical and maximum shelf-life; (iii) amendments to commercial packing technology and regimes have not impacted on the outcome; (iv) spores of non-proteolytic *C. botulinum* are identically and independently distributed throughout the retail unit packs of meat; (v) all the retail unit packs of fresh meat can be considered as a single homogeneous population of unit packs and that risk events arise independently from individual retail unit packs.

In 2017, total global sales of beef, lamb and pork amounted to 6.2×10^{11} ($10^{11.8}$) portions of 250 g, although not all of these presentations will have been as fresh chilled meat and/or VP/MAP. While this represents significant global sales of fresh chilled meat, without reports of foodborne botulism, there is greater uncertainty associated with estimating the level of protection. Importantly, however, these data support the assertion that the risk associated with fresh chilled red meat is small.

3.5. Challenge test study

Packs of chilled meat inoculated with spores of non-proteolytic *C. botulinum* and uninoculated packs were examined for gas production, exudate formation and meat discolouration at each sampling time. Packs started to lose their tight vacuum-packed appearance within 12 days (Table 6). Pork SM samples appeared unacceptable after 12 days at 8 °C due to the production of a thick, creamy exudate. Gas production in packs of Lamb LM was observed from day 12. By day 21, gas production was evident in packs of Pork LM. Both types of beef and Lamb SM were judged to look acceptable until day 35, when all samples appeared spoiled and smelled off (Table 6).

Type B botulinum neurotoxin was not detected in any of uninoculated packs of fresh chilled meat, but was detected in positive controls of microbiological broth from day 12 onwards (Table 7). All packs of inoculated chilled fresh meat contained < 40 pg type B toxin g^{-1} of meat, except for Pork SM at day 35 (Table 7). In one pack of Pork SM (short maturation time) at day 35, the concentration of type B toxin was *ca.* 450 pg toxin g^{-1} of meat. Type E botulinum neurotoxin was not detected in any inoculated or uninoculated packs of fresh chilled meat, but was detected in positive controls of microbiological broth (Table 7).

4. Discussion

Currently, UK industry as represented by the project consortium typically applies a chilled retail shelf life of up to 11–13 days to packs of fresh beef, lamb and pork, with a maximum of 23 days for beef, 27 days for lamb, and 18 days for pork. Production temperature regimes are consistent as they are subject to EU regulation enhanced by industry and commercial codes. The level of protection with respect to *C. botulinum* provided by existing practice in the UK for chilled, VP/MAP fresh meat held at 3 °C–8 °C is high, with 6.2×10^{10} ($10^{10.8}$) portions each of 250 g sold (between 1999-2005 and 2007–2017), with no associated cases of foodborne botulism. One important qualification is the assumption that existing commercial and consumer practice remain unchanged. If future changes are made to existing practice (including the fraction of packs sold with typical and maximum shelf-life), then these changes may affect the level of protection. The level of protection estimated for fresh chilled red meat is greater than that reported in other assessments of this type for defined luncheon meats, canned cured ham and sausages (Hauschild and Simonsen, 1985, 1986), and for cooked chilled food with a maximum shelf-life of 10 days at ≤8 °C (Peck et al., 2006). It is noted that the previously estimated levels of protection were considered acceptable (Hauschild and Simonsen, 1985, 1986; ACMSF, 2006). Furthermore, a considerable quantity of fresh chilled meat has also been sold globally without an association with foodborne botulism.

In the challenge test study, six examples of fresh meat were tested (two each of beef, lamb and pork; and for each species, meat of a short maturation time and a long maturation time). Meat samples were inoculated with spores of strains of non-proteolytic *C. botulinum* type B and E, and incubated at 8 °C to day 50 (beef) or day 35 (lamb and pork). All samples of beef and lamb were negative for type B and type E neurotoxin (i.e. < 40 pg type B toxin g^{-1} of meat and < 40 pg type E toxin g^{-1} of meat), and were visually spoiled at the end of the experiment. All samples of pork were negative for type B and type E neurotoxin (i.e. < 40 pg type B toxin g^{-1} of meat and < 40 pg type E toxin g^{-1} of meat) at day 25, with one sample of pork matured for a short period of time (Pork SM) positive for type B neurotoxin at day 35 (i.e. > 40 pg type B toxin g^{-1} of meat). Note that pork matured for a short period was deemed spoiled at day 12. Since the tested meats were selected by the BMPA to give a broad representation of the UK market (e.g. current UK maturation times), then it is likely that the findings of the challenge test experiment can be taken to be representative of that of fresh chilled beef, lamb and pork presently sold in the UK.

Several predictive models and some challenge test experiments indicate the potential for growth and neurotoxin formation by non-proteolytic *C. botulinum* in chilled foods (Peck et al., 2008). A literature review identified only eight previous reports of challenge tests for non-proteolytic *C. botulinum* on fresh meat at 7 °C or 8 °C. These include two publications (Moorhead and Bell, 1999; Hyytia-Trees et al., 2000), an electronic project report (Stringer et al., 2011), and five unpublished reports provided by industry members of the project consortium (summarised in Peck et al., 2019). However, interpreting previous challenge test data is problematic, with inconsistent results between and within challenge tests, and significant differences and potential limitations in the experimental protocols. Previous challenge tests often did not follow good practice (Anon, 2018), with potential limitations for example including one or more of (i) a high spore inoculum (e.g. 10^5-10^7 spores kg^{-1}): several orders of magnitude higher than reported for natural contamination of meat; (ii) a small number of *C. botulinum* strains (or one strain) (iii) a small mass of meat (e.g. 1 g); leading to a large surface area to mass ratio, not reflecting commercial practice; (iv) a failure to demonstrate an absence of botulinum neurotoxin formation, instead relying on the use of viable counts of sulphite-reducing

Table 6
Effect of storage time on visual spoilage in the fresh chilled meat challenge test.

Sample code		Detection of visual spoilage at specified storage time (days)								
		0	10	12	15	18	21	25	35	50
Beef SM	Inoculated	NS[a]	NS	NS	NS	NS	NS	NS	**S**[b]	**S**
	Uninoculated	NS	NS	NS	NS	NS	NS	NS	**S**	**S**
Beef LM	Inoculated	NS	NS	NS	NS	NS	NS	NS	**S**	**S**
	Uninoculated	NS	NS	NS	NS	NS	NS	NS	**S**	**S**
Lamb SM	Inoculated	NS	NS	NS	NS	NS	NS	NS	**S**	–[c]
	Uninoculated	NS	NS	NS	NS	NS	NS	NS	**S**	–
Lamb LM	Inoculated	NS	NS	NS	NS	NS	**S**	**S**	**S**	–
	Uninoculated	NS	NS	**S**	**S**	**S**	**S**	**S**	**S**	–
Pork SM	Inoculated	NS	NS	**S**	**S**	**S**	**S**	**S**	**S**	–
	Uninoculated	NS	NS	**S**	**S**	**S**	**S**	**S**	**S**	–
Pork LM	Inoculated	NS	NS	NS	NS	NS	**S**	**S**	**S**	–
	Uninoculated	NS	NS	NS	NS	NS	**S**	**S**	**S**	–

SM = short maturation time, LM = long maturation time.
[a] NS = No spoilage detected in any of the three replicate packs.
[b] S = Spoilage detected in at least one of the three replicate packs (see text for details).
[c] – = not tested (lamb and pork were not tested at day 50).

clostridia, despite foodborne botulism being an intoxication and evidence that an absence of growth may not equate to an absence of botulinum neurotoxin (e.g. Bell and Kyriakides, 2000; Brown and Gaze, 1990; Brown et al. 1991; Carlin and Peck, 1996; Hyytiä et al. 1999; Keto-Timonen et al. 2012); (v) uncertain provenance of the meat tested compared to fresh red meat presently sold in UK; and (vi) lack of suitable controls. Discrepancies in previous challenge test data inevitably mean that they do not always align with each other or the new challenge results, with more rapid toxin/growth sometimes reported previously and current commercial practice not always supported. However, caution should be used in interpreting previous challenge test data.

In conclusion, the exposure assessment and challenge test experiment both support the safety of current UK industry practices and the shelf-life of fresh chilled beef, lamb and pork held at 3 °C–8 °C. Additionally, in the challenge test experiment, botulinum neurotoxin was not detected within the product organoleptic shelf-life. UK industry typically applies a chilled retail shelf life at 3 °C–8 °C of up to 11–13 days to packs of fresh beef, lamb, and pork, with a maximum of 23 days for beef, 27 days for lamb, and 18 days for pork. Current production standards and shelf lives provide a high level of protection with respect to *C. botulinum*, with an estimated $10^{10.8}$ products marketed in the UK without an association with foodborne botulism. Current practice is also supported by the challenge test experiment. Important factors contributing to safety, may include low initial contamination with spores of non-proteolytic *C. botulinum*, the use of high hygiene standards during production, competing bacterial populations restricting non-proteolytic *C. botulinum*, low temperatures to the point of purchase by the consumer, and a limited shelf life.

Declaration of competing interests

None.

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Table 7
Effect of storage time on formation of botulinum neurotoxin by *C. botulinum* in the fresh chilled meat challenge test.

Sample code		Detection of botulinum toxin at specified storage time (days)								
		0	10	12	15	18	21	25	35	50
Beef SM	Inoculated	NT[a]	NT	NT	NT	NT	NT	NT	NT	NT
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
Beef LM	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT
Lamb SM	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	–[c]
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
Lamb LM	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
Pork SM	Inoculated	NT	NT	NT	NT	NT	NT	NT	**T**[b]	–
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
Pork LM	Inoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	–
Positive control	Inoculated	NT	NT	**T**	**T**	**T**	**T**	**T**	**T**	**T**
	Uninoculated	NT	NT	NT	NT	NT	NT	NT	NT	NT

SM = short maturation time, LM = long maturation time.
[a] NT = No botulinum toxin detected (i.e. all three replicate packs or both duplicate bottles for positive control contained < 40 pg type B toxin g^{-1} of meat and < 40 pg type E toxin g^{-1} of meat).
[b] **T** = Botulinum toxin detected in at least one of the three replicate packs or duplicate bottles for positive control (Pork SM sample at day 35 contained > 40 pg type B toxin g^{-1} of meat; positive control microbiological broth contained > 40 pg type B toxin ml^{-1} from day 12 onwards, and also > 40 pg type E toxin ml^{-1} from day 15 onwards).
[c] – = not tested (lamb and pork were not tested at day 50).

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